

# Test Instructions

## IMTEC-Cardiolipin-Antibodies IgM

### Enzyme Immunoassay for the quantitative Determination of IgM Antibodies against Cardiolipin

**REF** : TC 59081

Please read the instructions carefully before testing.

**Procedural precautions:**

- ▶ Do not use the reagents beyond the date of expiry.
- ▶ Never mix reagents from different test kits nor lots.
- ▶ Store reagents at 2-8°C.

#### 1. Clinical Use

Over the past years, the determination of autoantibodies against phospholipids, detected e.g. as anti-cardiolipin antibodies (aCL) or as lupus anticoagulant (LAC), has become increasingly important due to its association with arterial and venous thrombosis, tendency to abortion, neurological symptoms and signs as migraine and chorea, pulmonary hypertonia as well as thrombocytopenia and hemolytic anemia.

#### 2. Principle of the Test

The test is based on the immobilisation of cardiolipin in a biological active vesicle-like structure to a solid phase (polystyrene) and subsequent binding of the aCL. A better presentation of the antigenic epitope is achieved because of specially purified human  $\beta_2$ -glycoprotein I (the anti-phospholipid cofactor) was added and the sample buffer also contains  $\beta_2$ -glycoprotein I.

The bound antibodies are detected with a peroxidase-labeled secondary antibody that is directed against human IgM. After addition of substrate solution, a color stain develops. Its intensity is proportional to the concentration and/or the avidity of the detected antibodies.

The IMTEC standards are calibrated against the internationally accepted Sapporo standard (acc. to Koike et al, monoclonal antibody EY2C9).

#### 3. Materials Provided

-	<b>MTP</b> : Cardiolipin coated microtiter strips, (1x8), breakable	12 strips
-	<b>CAL</b> : standards, ready to use,	750 $\mu$ L
1	31.25 U/mL $\cong$ 6.3 ng/mL $\cong$ 7.1 MPL/mL	per vial
2	62.5 U/mL $\cong$ 12.5 ng/mL $\cong$ 14.2 MPL/mL	1 vial
3	125 U/mL $\cong$ 25 ng/mL $\cong$ 28 MPL/mL	each
4	250 U/mL $\cong$ 50 ng/mL $\cong$ 57 MPL/mL	
5	500 U/mL $\cong$ 100 ng/mL $\cong$ 114 MPL/mL	
	(all standards contain sodium azide and are inked according to concentration)	
-	<b>CONTROL</b> <b>-</b> : negative control serum, ready to use, contains sodium azide	1 vial 1 mL
-	<b>CONTROL</b> <b>+</b> : positive control serum, ready to use, contains sodium azide	1 vial 1 mL
-	<b>BUF</b> <b>WASH</b> <b>10x</b> : washing buffer concentrate (10x)	1 bottle 50 mL
-	<b>DIL</b> <b>SPE</b> : sample buffer, ready to use	1 bottle 100 mL
-	<b>CONJ</b> <b>a(hum IgM):HRP</b> : HRP-Conjugate, anti-human IgM, ready to use	1 vial 12 mL
-	<b>SUBS</b> <b>TMB</b> : TMB solution, HRP substrate, ready to use	1 bottle 12 mL
-	<b>SOLN</b> <b>STOP</b> : stop solution, ready to use, sulfuric acid, handle with care, corrosive!	1 bottle 12 mL

#### 4. Preparation of Reagents

**Attention! Allow the testkit and all its components to reach room temperature completely before executing it !**

**Please do not use any polystyrene vessels for handling of HRP conjugates.**

**If the test is performed automatically, we recommend the use of fresh conjugate each run and to discharge traces of old conjugate entirely. Remove washing buffer after washing steps completely.**

#### 4.1. Preparation of Washing Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part washing buffer concentrate [BUF] [WASH] [10x] with 9 parts distilled water. The diluted buffer is stable for 6 weeks stored at 2 – 8 °C.

#### 4.2. Stopping Solution, Sample buffer, Standards, Control Sera, HRP-Conjugate and TMB Solution

Stopping solution, sample buffer, standards, control sera, HRP-conjugate and TMB solution are ready to use. Used bottles should be closed carefully and stored at 4 - 8 °C. **Store TMB solution also protected from light.**

#### 4.3. Microtiter Strips

The strips are ready to use. Unused strips should be sealed in the lockable original bag at 2 - 8 °C.

#### 4.4. Preparation of Serum or Plasma

Use serum or plasma freshly collected or freeze samples at – 20 °C. Do not use samples, that are repeatedly thawed and frozen. Do not use serum or plasma inactivated by heat treatment at 56 °C. Allow the samples to reach room temperature (30 min). Dilute samples 1:100 with sample buffer [DIL] [SPE] (10 µL sample to 1 mL buffer).

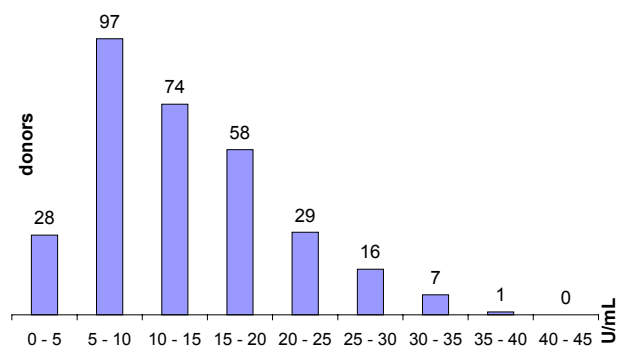
### 5. Test Procedure

- **Pipette 100 µL serum or plasma dilution** or undiluted standards [CAL], inked according to concentration, or control sera [CONTROL] + and [CONTROL] -, into each well, for blanks use [DIL] [SPE] instead of serum dilution, seal wells with adhesive foil.
- **Incubate for 1 hour** at room temperature (RT).
- **Rinse the wells 3 x** using at least 200 µL washing buffer per well.
- **Discard buffer and knock out residues** on an absorbent paper or cloth.
- **Pipette 100 µL HRP-conjugate** [CONJ] a(hum IgM):HRP into each well, seal wells with adhesive foil.
- **Incubate for 30 minutes** at RT.
- **Rinse the wells 3 x** using at least 200 µL washing buffer per well.
- **Discard buffer and knock out residues** on an absorbent paper or cloth.
- **Pipette 100 µL TMB solution** [SUBS] [TMB] into each well.
- **Incubate for 10 min** at RT in the dark. At room temperatures above 25 °C the substrate incubation could be shortened, but should never fall short of 5 minutes.
- **Pipette 100 µL stopping solution** [SOLN] [STOP] per well.
- **Measure at 450 nm** within the next 30 min after stopping.

### 6. Interpretation of Results

Calibrate measured absorbance against concentration/units of standards [CAL] (31.25 U/mL, 62.5 U/mL, 125 U/mL, 250 U/mL, 500 U/mL) in semi log. Determine the units of the examined sera or plasma samples from the standard curve directly. It is also possible to calibrate the test in Sapporo units (ng/mL, acc. to Koike et al. – related to a sample dilution of 1:100) or Louisville units (MPL/mL, acc. to Harris et al.) respectively. Using these, results above the respective cut-off values listed in the following table, are considered positive:

unit	cut-off IgM
U/mL	44 U/mL
Sapporo	8.8 ng/mL
Louisville	10 MPL/mL



A determination of Anti-Cardiolipin antibodies IgM in 310 blood donors shows the histogram.

To prove the functionality of the test, the determined value for the positive control serum [CONTROL] + is to be expected within the range labeled on the vial. The result of the negative control [CONTROL] - has to be lower than the cut-off value of the testkit.

#### Precautions

For in vitro diagnostic use only.

[IVD]

The human Control Sera and Standards in this kit have been prepared from blood donations which have been tested for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies and shown to be NEGATIVE.

However, as no known test can guarantee the absence of an infectious virus, all reagents and samples must be handled carefully and disposed of in accordance with local legislation.



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